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Review

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Glyphosate Effects on Plant Mineral Nutrition, Crop Rhizosphere Microbiota, and Plant Disease in Glyphosate-Resistant Crops

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ABSTRACT: Claims have been made recently that glyphosate-resistant (GR) crops sometimes have mineral deficiencies and increased plant disease. This review evaluates the literature that is germane to these claims. Our conclusions are: (1) although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate; (2) most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease; and (3) yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops.

KEYWORDS: *glyphosate, glyphosate-resistant crop, corn, cotton, soybean, maize, plant disease, mineral nutrition*

INTRODUCTION

Since the herbicide glyphosate (*N*-(phosphonomethyl)glycine) was commercialized in 1974, it has become the most widely used herbicide in the world, due largely to the wide scale adoption of transgenic, glyphosate-resistant (GR) crops after their introduction in 1996 (Figure 1). In GR crops, this relatively high use rate herbicide (commonly 0.5 to 2.0 kg/ha/application) is often used multiple times in a growing season. Use of other herbicides declined steadily, while glyphosate use increased in the three major GR crops (Figure 2). The increasing incidence of evolved, GR weeds,[2] as well as weed shifts to naturally glyphosate-tolerant weed species,[3] has resulted in increased use rates and numbers of applications of glyphosate, as well as other herbicides, per growing season in GR crops. Since its introduction, glyphosate has been considered a toxicologically and environmentally safe pesticide, due to its low mammalian toxicity, relatively short environmental half-life, and extremely low activity in soil due to its binding to soil minerals (reviewed by Duke et al.[4]). Furthermore, only green plants, some fungi, and a limited number of microorganisms have the target site, 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), of the herbicide. EPSPS is an enzyme required for synthesis of the essential aromatic amino acids phenylalanine, tyrosine, and tryptophan.

Glyphosate has several desirable properties that have contributed to its widespread use.[5] Glyphosate is a nonselective herbicide, that is, it can kill all plant species, although there is variation between species with regard to levels of natural tolerance. Glyphosate has little or no herbicidal activity in soil and, thus, is used only with foliar spray applications. Due to crop sensitivity, its use was limited in crop production prior to the introduction of GR crops, after which its use greatly expanded with the widespread adoption of these crops worldwide.

During its several decades of use over vast areas, no significant adverse secondary effects of the herbicide have been established, other than the intense selection pressure that has resulted in the evolution of GR weeds. In fact, its use in GR crops has been associated with several environmental benefits.[6,7] The topic of evolution of GR weeds has been dealt with in detail in many research papers and reviews.[8,9]

Several papers have been published recently that conclude that glyphosate adversely affects mineral nutrition in GR crops, leading to several adverse effects, including increased plant disease.[10−20] Others[21] have indicated that GR crops are more susceptible to plant diseases due to other mechanisms. This review addresses these concerns in the context of the available literature on glyphosate (ca. 8000 peer-reviewed papers according to SciFinder).

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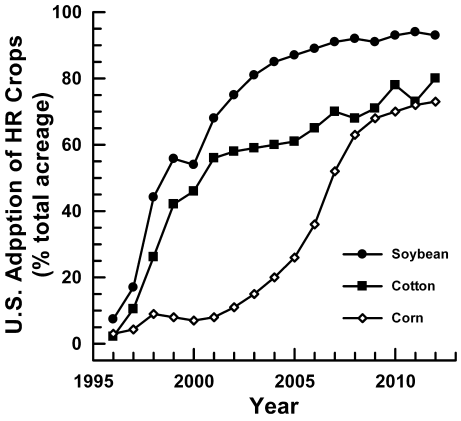

Figure 1. U.S. adoption of the three most widely grown, herbicide-resistant (HR) crops in the United States. Almost all HR crops during this period were GR crops. Data for each crop category include varieties with both HR as a single and stacked trait with insect resistance. Sources: 1996−1999 data are from Fernandez-Cornejo and McBride.[1] Data for 2000−2012 are available in the USDA, Economic Research Service data product, *Adoption of Genetically Engineered Crops in the U.S.*, Tables 1−3 (http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx, accessed September 12, 2012). Note that adoption data for 1996−1999 include HR corn and soybeans obtained using traditional breeding methods (not transgenic). The more recent data (2000−2011) excluded these varieties.

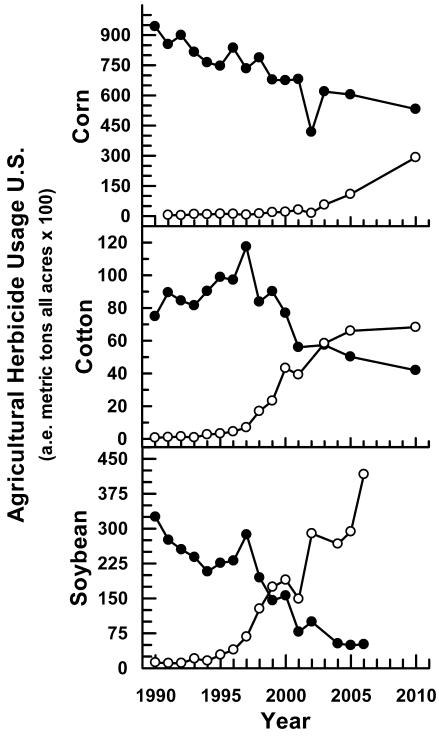

Figure 2. Agricultural herbicide usage in the U.S. Closed circles = all herbicides minus glyphosate, open circles = glyphosate only. Data from the U.S. Department of Agriculture, National Agricultural Statistics Service Data and Statistics site http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/ (accessed September 12, 2012).

GLYPHOSATE IN SOIL: BIOAVAILABILTIY, DEGRADATION, AND PERSISTENCE

In order to understand possible effects of glyphosate on mineral nutrition of plants, it is necessary to understand the processes that affect glyphosate in soil. It is also necessary to understand how glyphosate interacts with minerals in soil and with soil microorganisms.

Sorption/Bioavailability. Once glyphosate interacts with soil, whether applied directly to the soil surface, exuded from a plant root, or released from decomposing plant tissue, it is subject to various processes that control its environmental behavior and fate, including retention (sorption−desorption), transport, and degradation. Of these processes, sorption is arguably the most important as it controls the availability for degradation, plant uptake, and offsite transport. Sorption of glyphosate to soil has been extensively reviewed.[22−24] Because glyphosate is a small polyprotic molecule (pK_{a1} = 2.27, pK_{a2} = 5.58, pK_{a3} = 10.25[22]) with three polar functional groups and can be sorbed on minerals and organic matter, its sorption on soil as a whole is generally much greater compared to other pesticides, which are larger molecules with fewer functional groups and are primarily sorbed onto organic matter.

Glyphosate is primarily sorbed on variable-charge surfaces such as iron and aluminum oxides, aluminum silicates (allophone and imogolite), and goethite (α-FeOOH), and to a lesser extent on the Fe-oxide coatings of permanent charge minerals (illite, smectite, and vermiculite) and organic matter. The primary mechanisms responsible for sorption are ligand exchange and complex formation with mineral oxide surfaces. The magnitude of sorption increases with increased surface area of the minerals and decreased pH. The sorption onto the sorbent surface is fast initially for most of the glyphosate added to soil, which is then followed by slower sorption. Further details of these processes can be found in the above cited reviews and the references cited therein.

The magnitude of sorption has traditionally been characterized as the ratio of glyphosate bound to soil to that in solution for a single concentration (K_d) or at multiple concentrations (K_f and $1/n$ values from the Freundlich sorption isotherm). Sorption coefficients are often expressed on a soil organic carbon basis (K_{oc}, K_{foc}) to normalize values between different soils. Glyphosate is strongly bound to soil. For instance, regardless of soil properties in a cultivated prairie, glyphosate mean K_d was 108 to133 L kg^{-1} (K_{oc} = 10 900−14 900 L kg^{-1}), depending on landscape position, and these values were 100× greater than those for the commonly used herbicide 2,4-D.[25] In a study of 20 different soils, K_d ranged from 41 to 303 L kg^{-1} with a median value of 97 L kg^{-1}.[26] In column leaching experiments, coarse textured soils retained most all (85−95%) of the glyphosate applied despite the fact that higher than agronomic rates, 7.4−14.8 mg kg^{-1}, of glyphosate were used.[27]

After glyphosate is sorbed, it is not readily desorbed. Desorption is inversely related to adsorption, being small when sorption is great.[28] Depending on soil type, glyphosate is weakly desorbed, that is, 5−24% of initially sorbed glyphosate.[29] The strong adsorption of glyphosate to most soils, and its low desorbability, leaves little glyphosate in soil solution available for microbial degradation, interaction with trace metal cations, plant uptake, or offsite transport.

Sorbed glyphosate has been postulated to be released into soil solution upon addition of phosphate (PO_4^{3-}), which can compete with glyphosate for sorption sites on soil.[24] However, it appears there is only limited competition for sorption sites between glyphosate and PO_4^{3-}, even when much higher than agronomic rates of glyphosate are applied.[27] For soils, competitive sorption studies between glyphosate and PO_4^{3-}

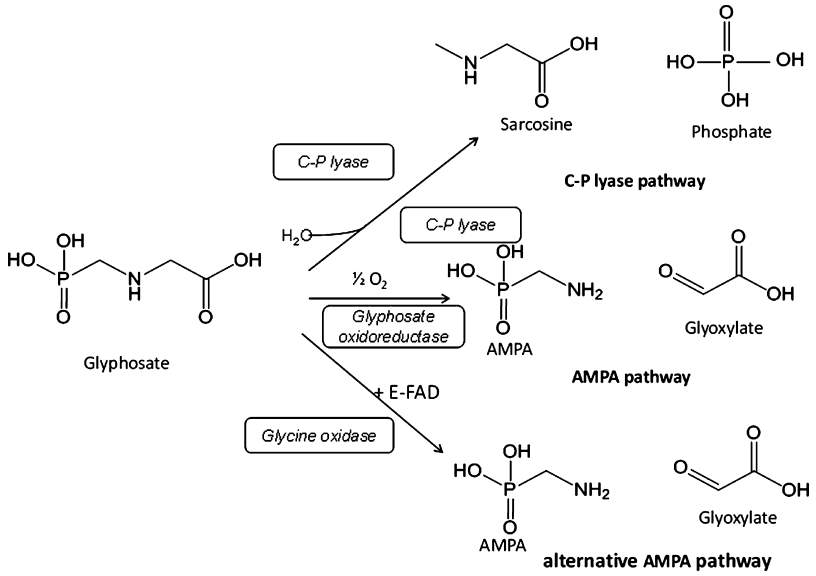

Figure 3. Catabolic degradation pathways of glyphosate.

showed that displacement of glyphosate by PO_4^{3-} was related to the amount of clays, CEC, and pH, but glyphosate was not easily displaced by PO_4^{3-} from the clays.[30] Even when sorption competition has been shown to occur, glyphosate still remains strongly sorbed. For example, increasing extractable P by a factor of 10 in soil, only decreased the sorption coefficient K_f from 215 to 106 L kg^{-1} in loamy sand and 154 to 84 L kg^{-1} in coarse sand soils.[31] As a result, solution concentration of glyphosate does not appreciably increase upon the addition of PO_4^{3-} at environmentally relevant P concentrations. Also, the competition between glyphosate and PO_4^{3-} (if it occurs) appears to be temporary; the same amounts of glyphosate and PO_4^{3-} were sorbed after ~7 days whether the compounds are present alone or together.[32] Therefore, it seems likely that glyphosate and PO_4^{3-} are specifically sorbed on to common as well as specific sites on various soil components.

Glyphosate can form chelates or complexes with micronutrient metal ions in solution. At physiologically relevant pH levels, and pH levels of most soils, Cu and Zn ions in solution can be relatively strongly complexed with glyphosate, whereas Fe, Ca, Mg, and Mn are complexed to lesser degrees.[22,33] Because of the ability of glyphosate to complex metal ions, glyphosate has been postulated to affect plant uptake of trace nutrients such as Mn^{2+} or Zn^{2+}. For plants grown in hydroponic solutions, mixed results for glyphosate effects on plants have been shown. In contrast, Andrade and Rosolem[34] reported that glyphosate did not affect Mn absorption and transport in GR soybean plants in the field. This topic is discussed in more detail in the section below on the effects of glyphosate on mineral nutrition of plants.

It is difficult to extrapolate hydroponic studies to field situations where there are numerous cations at varying concentrations that can form complexes with glyphosate, and where soluble metal-glyphosate complexes are subject to sorption processes on soil, as are glyphosate and metal ions. For example, the presence of Zn increased glyphosate sorption on two soils as a result of decreased solution pH resulting from Zn^{2+} exchanging with H^+ on the soil surface.[35] In bioassay experiments using tomato plants and white spruce seedlings, soils containing saturated solutions of glyphosate-metal complexes had little or no effect on the plants.[33] In a recent study of micronutrient accumulation in soybean grown using standard agronomic practices in Indiana, results showed that while there were differences in accumulation of micronutrients between cultivars, there was no consistent effect due to glyphosate treatment.[36]

Micronutrient metal concentrations in soil solutions can vary spatially and temporally. For example, in five woodland sites, the mean soil solution concentrations of total Mn and Zn were 69 and 1.8 μmol L^{-1}, respectively, while in grassland sites, total Mn concentrations varied by a factor of 6 in a clay soil and a factor of 2 in a sandy soil.[37] In a soil toposequence under natural vegetation, total Mn concentration in soil solution varied by a factor of 900, depending on topographic soil position.[38] In addition, the metal cations in soil solution are not necessarily free ions; they can form complexes with dissolved soil organic matter and other ligands. For example, in a study of 15 agricultural soils, total soil solution concentrations of Cu ranged from 0.023 to 1.03 μmol L^{-1}, with free Cu^{2+} comprising 7−73% of total dissolved Cu.[39] In the same soils, total Zn solution concentration ranged from 0.4 to 43 μmol L^{-1}, with free Zn^{2+} comprising 47−99% of total dissolved Zn. Glyphosate can only compete with other soil ligands and sorbent surfaces for free metal ion activity, with most glyphosate being adsorbed by the soil rather than remaining in the soil solution where it can complex with metal ions.

In spite of the wide range of micronutrient cation concentrations in soil solutions, their concentrations are much greater than would be found for glyphosate in soil solutions. Using a glyphosate application rate of 1 kg ha^{-1} (soil concentration = 0.75 μg g^{-1}, assuming incorporation to a depth of 10 cm and a soil bulk density of 1.33), and an average soil K_d = 100, the amount of glyphosate in soil solution would be 0.044 μmol L^{-1}, which is much smaller than typical Mn^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{3+} concentrations found in soil solutions from agricultural soils. Under agricultural production, concentrations of, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{3+} in soil solutions of Holtville (Typic Torrifluvent) and Altamont (Typic Chromoxerert) soils would be on average 480×, 220×, 80×, and 310× greater than the glyphosate in solution, respectively.[40] Therefore, free cation concentrations, such as Mn^{2+}, would not be reduced appreciably by glyphosate addition to soil, even at the highest

recommended application rates and assuming all the glyphosate in solution formed a chelate with Mn. Furthermore, glyphosate degrades rapidly (see degradation section below), whereas although micronutrient concentrations in soil can fluctuate temporally during the year, micronutrients do not degrade.

GLYPHOSATE DEGRADATION, PERSISTENCE, AND LEACHING

Biological Degradation Pathway. The degradation of glyphosate in soil has been extensively documented.[24,41] The primary degradation pathway is the cleavage of glyphosate by glyphosate oxidoreductase to glyoxylate and AMPA (aminomethylphosphonic acid) (Figure 3), with the latter subsequently degraded to methylamine and inorganic phosphate by C−P lyase enzymes. Both glyoxylate and methylamine can support the growth of microorganisms. Alternatively, the transformation of glyphosate to AMPA and glyoxylate can also be performed by glycine oxidase.[42]

A second degradation pathway is the cleavage of inorganic phosphate from glyphosate by C−P lyase producing the sarcosine metabolite (Figure 3). Sarcosine is further degraded into formaldehyde and glycine, which are utilized by a wide variety of soil microorganisms. Soil microorganisms utilizing the sarcosine pathway have been isolated and characterized,[43] including members of the soybean root symbionts, the Rhizobiaceae.[44] Soil fungi also degrade glyphosate, and AMPA was reported as a metabolite.[45] Since some microorganisms are sensitive to glyphosate (see this review), the degradation of glyphosate in situ represents the activity of the glyphosate-degrading microbial community modulated by relative resistance or sensitivity to the herbicide.

^{14}C-Glyphosate Fate Studies. Several studies have utilized ^{14}C-labeled glyphosate to examine the fate of glyphosate in soil. These methods are useful because they provide an integrated assessment of glyphosate degradation by measurement of $^{14}CO_2$ production (mineralization) and the incorporation of glyphosate and glyphosate degradation products into soil organic matter and biota (bound ^{14}C residue). The $^{14}CO_2$ produced from microbial degradation after glyphosate addition to soil is variable, ranging from ≤10% in 332 d,[46] to <10−40% in 96 d,[47] to 50−70% in 35 d,[48] to >70% in 140 d,[49] and depends on soil type and ^{14}C-label location (phosphomethyl-^{14}C versus aminomethyl-^{14}C). The production of $^{14}CO_2$ begins after addition of glyphosate to soil without a lag period, showing that microorganisms with the capacity to degrade glyphosate are present in most all soils. The studies conducted with ^{14}C label in the phosphonomethyl carbon show degradation of the AMPA metabolite. The variability in the amount of $^{14}CO_2$ produced from glyphosate degradation in soil is likely due to the variability in the population of glyphosate-degrading microorganisms present in soil (the glyphosate-degrading microbial community in soil has not been fully characterized) and their biological activity and to competing sorption and binding processes.

In addition to the direct application of glyphosate to soil, any glyphosate remaining in GR crop residues will be released to the soil as those crop residues degrade.[50] At 35 days after treatment, glyphosate residues within corn leaves mineralized more slowly (61%) than glyphosate applied directly to soil (77%).[51]

The use of GR crops allows for multiple applications (commonly two or three) of glyphosate within a field each growing season. ^{14}C-labeled glyphosate has also been used to determine whether repeated applications of glyphosate can affect glyphosate degradation in soil. Repeated applications reduced the rate of glyphosate mineralization by 28% from the initial application to the fifth application in a 10-wk period (Figure 4). However, there was no difference in the rate of ^{14}C-

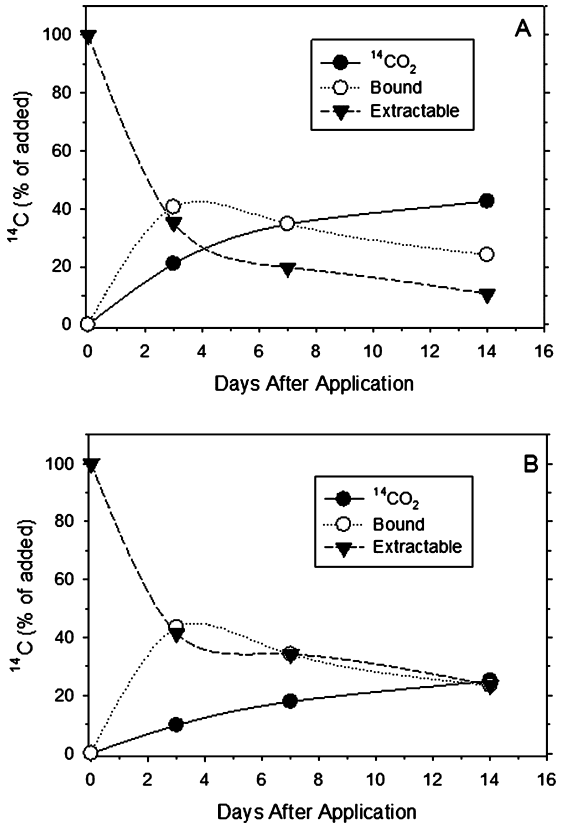

Figure 4. Distribution of ^{14}C after the (A) first or (B) fifth glyphosate application to silt-loam soil The sequential glyphosate applications were made at 2 week intervals. The extractable fraction includes glyphosate and its transformation products extracted with 0.1 M NaOH.[52].

glyphosate mineralization between one, two, three, or four glyphosate applications.[52] While the rate of mineralization was decreased after the fifth application relative to the first, it was not reduced relative to the second, third or fourth application. In a similar experiment, Andréa et al.[53] found that the initial, immediately mineralizable glyphosate decreased after sequential applications as compared to the initial application. However, after the initial mineralization, the rate of mineralization was the same. For instance, the differences in the total amount of $^{14}CO_2$ detected 8 wks after treatment and the immediately mineralizable glyphosate were 16 and 19% after 1 and 4 sequential applications, respectively. Weaver et al.[54] reported greater mineralization (% of applied) from glyphosate added at 1× the field rate (47 mg glyphosate/kg soil, assuming 0.84 kg/ha application distributed in the surface 2 mm of soil) than at the 3× rate of application. However, on a mass basis more glyphosate was degraded in the soil receiving the 3× application. These studies show that repeated glyphosate applications are unlikely to severely reduce the ability of the soil microbial community to metabolize glyphosate. Broad spectrum measures of microbial activity (respiration and

enzyme activities) and community structure show inconsistent or no response to glyphosate use (see Effects on Rhizosphere Populations and Community Structure section).

In addition to ^{14}C-glyphosate being mineralized, a portion of the ^{14}C-labeled glyphosate or its metabolites is converted to microbial biomass and some remains unextractable from soil. The amount of bound ^{14}C-residue formed depends upon the molecular location of the ^{14}C-label, the soil interaction, and the extraction methods used. Mamy et al.[49] compared the bound residue formed from several pesticides and found that glyphosate-bound residues generally accounted for less than 20% of the initial ^{14}C added, equivalent to trifluralin, but less than those formed after application of the ^{14}C-labeled herbicides metazachlor, metamitron, and sulcotrione. Weaver et al.[54] applied glyphosate equivalent to 1× or 3× recommended field rates, and less than 10% of the ^{14}C was present in bound residues at 42 days after application. After four[53] or five[52] sequential applications at 14-day intervals, less than 30% of the ^{14}C from applied glyphosate was present as bound residue in the soil, and there was no difference in bound residue formation after the first or the fifth application. However, greater accumulation of bound residues would occur from multiple applications than a single application. Simonsen et al.[55] assessed the bioavailability of bound residues formed after a single glyphosate application to plants by incubating ^{14}C-glyphosate in soil for 6.5 months, followed by planting canola or barley. Glyphosate was not detectable in soil at planting, and after 41 days of plant growth only 0.006% of the applied ^{14}C was detected in the plants.

Persistence and Leaching in Field and Lysimeter Studies. Field dissipation rates of glyphosate are affected by soil properties, method of application, and environmental conditions such as moisture and temperature, and therefore are extremely variable.[24] Field studies often result in longer estimated times to 50% dissipation (DT_{50}) as compared to laboratory studies, which are generally conducted under optimum conditions for degradation. In one field study, under the application and weather conditions that prevailed (which resulted in water-saturated soils), no glyphosate was detected 24 h after treatment, with a trace amount detected in one replicate soil sample after 1 year.[56] Warm temperatures at the time of and in the season before the application are thought to explain the fast degradation rate in the water-saturated soil samples. In a comparison among glyphosate treatments between a forest floor and mineral soil, the DT_{50} times for glyphosate were 12 and 10 days for the forest floor matrix and mineral soil, respectively.[57] Simonsen et al.[55] measured a 9-day DT_{50} for glyphosate and 32-day DT_{50} for AMPA in soil. In an agronomic field study, glyphosate dissipation in the surface soil was rapid (DT_{50} = 25 days) and only 10% of applied chemical was present at 34 days after application.[58] In another field study, glyphosate had an estimated DT_{50} of 45−60 days, with total soil residues of glyphosate accounting for 6−18% of initial chemical at 360 days after application.[59] Bergström et al.[60] reported a 110 to 151 day DT_{50} for glyphosate in a clay soil and attributed the long persistence to adsorption (K_f > 118).

As a result of strong sorption and slow desorption, some glyphosate residues tend to stay in the surface soils through the growing season. For example, of the initial amount of glyphosate added to a clay soil, 59% (glyphosate + AMPA residues) remained primarily in the surface soil 748 days after application, despite large amounts of precipitation after application.[60] Also, only 0.009 and 0.019% of the initial amount of glyphosate added leached from the sand and clay soils, respectively, during the study period. No leaching of AMPA occurred in the sand, whereas 0.03 g ha^{-1} leached in the clay soil.

Longer glyphosate persistence in colder climates has been observed. The DT_{50} time of glyphosate was generally <5 months in Swedish railway embankments.[61] In Northern climates with seasonally frozen soils, field studies have shown clear overwinter persistence for glyphosate. After glyphosate applications in June and July, about 10−20% of applied glyphosate was detected in the subsequent June in two field sites, demonstrating that the time for 90% (DT_{90}) dissipation of glyphosate was about 11 months.[62] Similar overwinter persistence was observed in agricultural fields in southeast Finland.[63] Under warmer climates, glyphosate did not persist past the growing season, even after 15 consecutive annual applications.[64]

After either pre-emergence or postemergence applications of glyphosate, the distribution of residues is nonuniform in soils and is more concentrated near the surface of the soil. Almost two years after application to a tilled soil in an outdoor lysimeter, glyphosate accounted for 1% and AMPA for 19% of the applied glyphosate in the 0−10 cm depth increment.[65] In the 10−20 cm depth increment, glyphosate was not detectable, and AMPA accounted for 5% of the applied herbicide. Deep (>1 m) leaching of glyphosate has been observed, but concentrations in leachate were <0.07 μg L^{-1}.[23,60,66] This was attributed to movement in macropore flow, rather than leaching through the bulk soil. Deep movement of glyphosate might be also expected via translocation of the herbicide sprayed on to foliage of crops and weeds to their roots, particularly resulting from glyphosate applications later in the growing season.

Multiple applications of glyphosate in GR-cropping systems would (1) increase the risk of carryover, especially in regions where soils are seasonally frozen for extended periods and (2) increase the risk of leaching to tile drains or groundwater. Multiple applications of glyphosate increase the time that bioavailable glyphosate is present in soil. Also, plant interception of glyphosate in the field may lead to a delayed release of glyphosate into the soil following foliage decomposition. The degree of metabolic degradation of glyphosate in plants[41] would influence how much glyphosate is released into soil by degradation of plant material. Such delayed releases would increase DT_{50} times. Both of these processes have been investigated in laboratory experiments, but corresponding field studies are not yet available. Any increased persistence is potentially important in cropping systems where glyphosate-sensitive (GS) crops closely follow the GR-crop. The risks associated with planting highly sensitive crops shortly after (<3 days) glyphosate applications were known long before the advent of GR-crops.[67,68]

GLYPHOSATE AND MINERAL NUTRITION OF PLANTS

During the course of their action on susceptible plants, herbicides eventually affect almost every physiological and biochemical process, including mineral nutrition. Thus, glyphosate would be expected to affect mineral nutrition of GS plants at herbicidal doses, but not of GR plants at the same doses. Recent reports of mineral deficiencies in GR crops after glyphosate application were linked with claims of increased susceptibility to plant diseases.[11,15,16,6] There are conflicting papers on the effects of glyphosate on mineral nutrition on GR

crops. This is a complex topic that, for clarity, we have separated into the aspects listed below.

Phytotoxicity of Metal Chelators. Many natural metal chelators such as organic and amino acids are found in plant cytoplasm and xylem and phloem fluids. Citrate is an important chelator of Fe in xylem fluid, while some amino acids chelate metals in the cytoplasm where the higher pH favors chelation by amino acids compared to organic acids.[70] Synthetic chelators have been used in agriculture since 1950 to supply Fe or Zn to plants, and more recently to induce phytoextraction of soil metals by plants.[71] Adding high amounts of strong chelators such as EDTA (ethylenediaminetetraacetate) to soils causes sorbed metals to be released from soil and metal chelates to be formed, making the metal mobile. In order for added chelating agents to be effective in increasing metal uptake, huge amounts have to be applied to soil. For example, induced phytoextraction of Pb required the addition of 10 mmol of EDTA kg^{-1} of soil which would cost over \$30 000 ha^{-1}.[71] EDTA was only effective when, after binding other metals in the soil, there was some free EDTA which attached to the root membranes, causing them to become leaky. High uptake of PbEDTA kills plants quickly. The added EDTA, however, also causes metal leaching, and use of EDTA to induce phytoextraction of soil metals is not allowed in the open environment.[72] Occasionally excessive fertilizer rates of FeEDTA or ZnEDTA cause phytotoxicity in the field or greenhouse.

Our experience with metal chelation in soils and metal chelate injury of plants provides insight into whether glyphosate would be expected to affect plant uptake of micronutrient cations. If low concentrations of EDTA or similar strong chelators are added to soils, they can promote uptake of strongly adsorbed metals because the dissolved metal-chelate can move metals from soil particles to the root membranes, circumventing the diffusion limitations of metal uptake. Thus, in general, addition of kg per ha levels of glyphosate might be expected to increase element uptake if glyphosate were a strong chelator. However, glyphosate is a relatively weak metal cation chelator compared to EDTA[73−75] (Table 1). In general, none of the research on chelating agent effects on metal uptake would indicate that a weak chelator such as glyphosate would reduce or increase uptake of micronutrient cations from soil.

Table 1. Logarithms of Metal Chelate Formation Constants for Representative Chelators and Glyphosate[a]

element	EDTA	citrate	glycine	glyphosate	AMPA
Ca^{2+}	12.4	4.9	1.4	3.25	1.62
Cd^{2+}	18.2	5.0	4.4	7.29	5.14
Co^{2+}	18.2	6.3	5.1	7.23	4.58
Cu^{2+}	20.5	10.9	8.6	11.93	8.09
Fe^{2+}	16.0	6.1	4.3	6.87	
Fe^{3+}	27.7	13.2	10.9		
Mg^{2+}	10.6	4.9	2.1	3.31	1.94
Mn^{2+}	15.6	5.0	3.7	5.47	3.62
Ni^{2+}	20.1	6.6	6.2	8.10	5.3
Zn^{2+}	18.2	6.1	5.4	8.74	4.91

[a]Although protonated and deprotonated chelates also occur, the 1:1 metal-ligand chelates are listed for comparison using data from the Program Geochem-PC.[70] Values for EDTA, citrate and glycine are for 0 ionic strength, while the values for glyphosate are 0.1 M ionic strength.[74] Values from AMPA are for 0.1 M ionic strength.[76] Equilibria depend very strongly on solution pH and the pKa values for the different proton binding functional groups of a chelator molecule.

Ratio of Glyphosate to Mineral Content in Glyphosate-Treated Plants. Examination of glyphosate levels in glyphosate-treated GR soybean seeds at maturity[77] and mineral levels in soybean seed[78] shows that on a molar basis the metal:glyphosate ratio can be from almost 10 000 times more Mn to around 100 000 times more minerals such as Mg or Ca compared to glyphosate. Comparing glyphosate content of leaves of glyphosate-treated GR soybean[79] with recently measured mineral content of GR soybean leaves,[80] the ratios are smaller (ca. 300 for Ca, 30 for Fe, 20 for Mn, and only 2 for Cu), but the ratio of total metal atoms to glyphosate molecules is close to 1000. Even if a substantial fraction of the minerals in the plant tissue were unavailable to glyphosate due to chelation with other compounds, sequestration, or other means, the ratio of mineral cations to glyphosate anions would still be very large. These large ratios do not support the view that the chelator properties of glyphosate would interfere substantially with plant mineral nutrition *in planta*. Furthermore, at very high *in vivo* concentrations of glyphosate in the plant phloem, glyphosate has been calculated to be unable to effectively compete for Fe^{-2+}, Fe^{-3+}, Ca^{-2+}, Mn^{-2+}, Mg^{-2+}, Cu^{-2+}, and Zn^{-2+} with biological chelating agents.[81]

Effects of Glyphosate on Mineral Content of Soils. Soil mineral status can affect plant mineral status. Reduction in soil Mn concentrations due to glyphosate use has not been demonstrated. In practice, glyphosate which reaches soil is strongly adsorbed by Fe and Mn oxides and organic matter.[27] When glyphosate is bound by soil, it can be abiotically degraded[82] in addition to the biodegradation pathways discussed earlier. Other studies tested whether glyphosate reaching soil would cause leaching of soil metals. Barrett and McBride[83] tested leaching of metals in response to glyphosate application for several soils and found that leaching occurred only with soils highly contaminated with metals and only with high rates or repeated applications of glyphosate. This outcome is predictable from the weak chelation of metal ions by glyphosate. In contrast with some descriptions of glyphosate as a strong chelator, the stability constants of glyphosate, EDTA, and citric acid with common micronutrient ions show that glyphosate is a weak chelator[73−75] (Table 1). The fact that the relative concentrations of metal cations in soil are several orders of magnitude greater (in terms of moles of metals per ha vs moles of glyphosate per ha) than the highest concentrations of glyphosate that could be expected (discussed in detail in a previous section), significant effects of glyphosate on soil mineral content or availability to plants are highly unlikely.

Effects of Mineral Ions on Glyphosate Efficacy. From the earliest days of glyphosate use, it was known that using water containing high levels of metal ions would significantly reduce the efficacy of the herbicide, presumably because the precipitated or chelated herbicide is not taken up by target plants as well as the free glyphosate anion and/or precipitation of glyphosate:mineral complexes (reviewed by Duke,[22] Sundaram and Sundaram,[33] and Nilsson[84]). If metal cations are present in a tank mix solution, and pH is raised by addition of microelement fertilizer or by hard water, precipitation of glyphosate reduces the plant uptake of glyphosate, thereby significantly reducing its herbicidal effectiveness. The solubility of 1:1 metal/glyphosate complexes decreases in the order of Mg ≈ Ca > Mn > Zn > Cu > Fe.[33] In a 3 year study, Chahal et al.[85] found Ca, Mn, and Zn ions to reduce glyphosate efficacy on a variety of weeds when included in a tank mixture. Several researchers have shown that separate application of Mn

fertilizer and glyphosate caused no effect or interaction, and recommend careful consideration of tank mixes.[86−88] EDTA, being a stronger chelator than glyphosate, reverses the reduction of glyphosate herbicidal efficacy by metal cations in spray tanks.[89] Farmers have been advised to not spray glyphosate with micronutrient plant nutrition supplements unless the metal is chelated with a strong chelator such as EDTA or EDDHA. Thus, studies on effects of glyphosate on mineral nutrition of plants should not be conducted with combined spray solutions of minerals and glyphosate. In short, a finding of metal ion precipitation of glyphosate in a tank mix is not relevant to questions raised about chemical interactions between glyphosate and micronutrients in plants or soils.

Glyphosate Effects on Mineral Nutrition in GS Plants. Because glyphosate is a metal ion chelator, there was speculation decades ago that this might be related to the mode of action of the herbicide. However, the finding that GR crops with only a change in their EPSPS are about 50-fold less sensitive to glyphosate than similar GS crops[79] indicated that mineral nutrition is not involved in the mode of action of glyphosate. Further evidence of this is the recent evolution of GR Palmer amaranth (*Amaranthus palmeri*) biotypes that have multiple copies of the GS EPSPS gene.[90,91] The greater the number of copies of the gene, the more resistant these plants are. If chelating Mn or any other mineral was significantly involved in the mode of action of glyphosate, this would not be the case.

Glyphosate can impede absorption and translocation of calcium and magnesium in GS plants (reviewed in Duke[22]). Nilsson[84] found glyphosate to stimulate the accumulation of Fe^{3+} in GS plants, while impeding movement of Zn^{2+} to the same sites. This result supports the finding that subtoxic levels of glyphosate stimulate growth of iron-deficient wheat.[84] Nilsson[84] found no effects of glyphosate on Mn, Zn, or Cu content of GS wheat leaves. Eker et al.[92] reported that glyphosate reduced uptake and translocation of Mn and Fe in GS sunflower. Likewise, Tesfamariam et al.[93] found reduced Mn in GS sunflower treated with glyphosate. Foliar-applied glyphosate to GS soybean seedlings reduced uptake and translocation of Mg^{2+} and Ca^{2+}, reduced tissue Ca content, and altered cellular Ca distribution.[94] Cakmak et al.[95] found reduced levels of Ca, Mn, Mg, and Fe in seeds and leaves of glyphosate-treated, GS soybean. In studies with GS *Festuca* spp., Ca, Mg, Mn, and Fe were most reduced by glyphosate treatment compared to other minerals.[96] Such effects are readily explained by the known effect of glyphosate on root growth and function in GS plants. Glyphosate from foliar sprays is rapidly translocated to roots, where it strongly inhibits root growth and other processes. Mineral uptake is highly dependent on physiological regulation by growing young roots. Nearly all of the multivalent metal cations are absorbed for translocation to shoots by young roots.[97−99]

Bellaloui et al.[20] reported reductions in plant shoot Fe due to glyphosate application, resulting in chlorosis in both GR and GS soybean cultivars. The authors correlated the effects on Fe content with effects on root ferric reductase activity, however, the methods used for measuring ferric reductase activity were inappropriate. Roots grown in soil were removed, washed, and used in a bioassay of FeEDTA reduction. Broken roots, loss of fine roots and root hairs, and the presence of soil in the assay mixture confounds the measurement. Ozturk et al.[100] found inhibitory effects of glyphosate on root ferric reductase in iron-deficient GS sunflower. However, no *in vitro* effect of the herbicide on the enzyme was reported to determine whether it was a primary or secondary effect.

High rates of phosphate fertilizer have been reported to remobilize small amounts of glyphosate bound to soil.[101] These low soil solution concentrations of glyphosate were phytotoxic to a GS soybean cultivar on most soil types, but stimulated plant growth (hormesis) on one soil type. Hormesis (the stimulatory effect of a toxin at subtoxic concentrations) at low glyphosate doses is a well-established phenomenon (e.g., Velini et al.[102]). However, the Bott et al.[101] experiment has no relevance to practical field environments, as the researchers applied extreme rates of dissolved superphosphate to the surface of glyphosate-amended soils and planted the seeds immediately. Fertilizer P rates are usually applied in bands below and to the side of seeds to prevent adverse effects on seed germination. Considering that the 240 mg P kg^{-1} highest rate of P application would cause the amount in the surface 1−2 cm of the potted soil to be 10−20 times higher than normally found in field applications of P, one should not extrapolate from the results with the high rates used in this study. It is questionable even whether the low rates, where no adverse effects were observed, are relevant to understanding glyphosate in the environment.

The studies discussed in this subsection were done on GS plants, so separating secondary effects of inhibition of EPSPS and effects via any other mechanism is impossible. It may be that some of the confusion regarding glyphosate effects on mineral nutrition of GR crops is due to studies on GS plants that cannot be extrapolated to GR plants.

The Cause of "Yellow Flash" Symptoms in GR Plants. As mentioned above, GR crops are highly resistant to glyphosate, with resistance factors (I_{50} ratios between GR and susceptible crops) of about 50 for both GR canola and GR soybean.[79] No effects on growth of GR crops are normally seen at the highest recommended field rates of glyphosate. Under some environmental conditions with some cultivars, transient "yellow flash" symptoms in GR soybeans are seen 5 to 20 days after glyphosate application (Figure 5). Yellow flash has been attributed to the rapid metabolism of glyphosate to the weakly phytotoxic AMPA and not to mineral nutrition effects.[103−108] GR crops are not necessarily resistant to AMPA, as its mode of action is not the same as that of glyphosate. The yellow flash

Figure 5. Example of "yellow flash" in GR soybeans sprayed with glyphosate in Illinois.[103]

effect is temporary and does not reduce yields, nor have yellow flash symptoms been shown to be due to disease incidence in soybean. This yellowing and interveinal chlorosis of rapidly growing young leaves in soybeans experiencing yellow flash could be confused with symptoms of Fe or Mn deficiencies. However, yellow flash symptoms are not accompanied by effects on Mn status of the plant or on Mn uptake or distribution by the plants.[109] Yellow flash symptoms have not been reported in GR crops other than soybean, perhaps because sufficient levels of AMPA to cause such symptoms do not accumulate in other GR crops or there is insufficient sensitivity of these crops to AMPA. Little is known of AMPA in GR crops, including its mechanism of phytotoxicity.[41]

Effects of Glyphosate on Mn in GR Crops. Huber[110] suggested that use of glyphosate in production of GR soybean leads to Mn deficiencies by reduction of Mn uptake and/or translocation efficiency, changing soil/rhizosphere microbiology, or modifying the form or availability of Mn in the environment. Dodds et al.[111,112] noted that GR-soybean cultivars showed lower yield, stronger yellowing symptoms, and lower foliar Mn on a Mn marginal or deficient soil than two conventional cultivars (non isolines). Application of microelements had no effect on either soybean type. It now appears that they observed that the GR-cultivar was inherently less able to obtain soil Mn than the conventional cultivars.[113] Mn deficiency can occur in soybeans grown on low Mn soils such as the Lake Plain soils in the Midwest, and the Coastal Plain soils on the east coast of the United States. If these soils are limed, Mn becomes much less phytoavailable and soybeans may suffer severe chlorosis and yield reduction until foliar Mn sprays are applied or soil pH is lowered.[114−116] Genetic variation for susceptibility to Mn deficiency exists in soybeans (e.g., Graham et al.[115]). Soybean cultivars for areas with low phytoavailable soil Mn have been developed, and farmers are advised to plant more Mn deficiency-resistant cultivars on such soils. As breeders worked to solve this susceptibility problem (much like the case of Fe chlorosis susceptibility of the early GR soybean cultivars; see below), improved cultivars with the GR trait were also resistant to Mn deficiency. This genetic variation in resistance to Mn deficiency among soybeans occurs because roots change the microenvironment in their rhizosphere to reduce Mn oxides to the soluble Mn^{2+}, or reduce chelated Mn^{3+} with fulvic acids to promote uptake by the roots. Local acidification of the rhizosphere may also improve Mn uptake by cultivars resistant to Mn deficiency. Plants also up-regulate metal ion transporters in their young roots to better absorb the free Mn^{2+} in the rhizosphere.

Experiments have been conducted in the field at multiple locations over multiple years which found that there was no appreciable susceptibility to Mn deficiency or need for Mn fertilizer to grow GR-soybean cultivars.[36,117] Several field trials have shown that GR-soybeans are not commonly experiencing Mn deficiency.[80,86,103,113,117] Unfortunately, no study has been reported on soils which caused clear Mn deficiency in soybeans in the absence of glyphosate so that any interaction with glyphosate use could be measured.

There are several peer-reviewed journal claims of effects of glyphosate on mineral nutrition in GR soybean. Bott et al.[10] reported that in the absence of glyphosate, a hydroponically grown GR soybean cultivar accumulated more Mn than did a GS cultivar, but the two lines were not near isogenic, making interpretation of the data impossible. In addition, when both types of soybean were grown with low Mn supply, there was no effect of glyphosate on shoot concentration of Mn or growth. At very high application rates of glyphosate, Mn concentrations in the tissue of the GR cultivar were reduced about 50%. There were no effects of glyphosate on Mn and Fe content of plant tissues when the plants were grown in two different soil types, although there was a reduction in insoluble foliar Zn in one of the soil types. This tests whether the low molecular weight soluble chelates were formed in the tissues as occurs with excessive EDTA. Taken together, the data of this study show no adverse effect of glyphosate on Mn uptake or translocation in GR-soybeans. Zobiole et al.[12,14,15,17,19] reported that glyphosate treatment reduced essential minerals (Mg, Mn, etc.) in GR soybean tissues. They also reported dramatic reductions in photosynthesis associated with these reductions,[12,19] a result that is difficult to reconcile with the high and increasing yields of these crops (see section on yields below). In a more extensive study, Cavalieri et al.[118] examined the effects of 0.96 kg ha^{-1} glyphosate from six different commercial formulations on N, P, K, S, B, Ca, Mn, Mg, Fe, Zn, and Cu in two GR soybean cultivars in the greenhouse. The results were equivocal, with both decreases and increases in metals, depending on both cultivar and glyphosate formulation. There was no clear pattern, other than reduced levels of both metal and nonmetal elements as well as plant growth by one formulation on one of the cultivars, suggesting that something other than glyphosate was involved.

Comparing near-isolines of soybeans, Loecker et al.[117] found no effect of the GR transgene of GR soybean on Mn uptake or response to Mn in the absence of glyphosate. Rosolem et al.[107] found no effects of foliar application of glyphosate on Mn absorption, accumulation, or distribution in GR soybeans. Similar results were reported by Andrade and Rosolem.[34] Serra et al.[119] found no effect of glyphosate doses up to 2.5 kg/ha on Cu, Mn, and Zn uptake by GR soybeans, while Fe uptake increased at this high dose. No effects of glyphosate on translocation of these metal ions were seen up to 2.5 kg/ha. In this study, exogenously applied Mn had no effect on any responses to glyphosate. Lundry et al.[120] found no effects of glyphosate on mineral nutrition in GR soybean seeds, compared to an untreated near-isogenic soybean line, indicating no effect from the EPSPS transgene or from glyphosate. Henry et al.[36] found no glyphosate-induced deficiencies in macronutrients (N, P, K, S, Mg, and Ca) or micronutrients (B, Zn, Mn, Fe, Cu, and Al) in second generation GR soybeans. The application of glyphosate to GR soybean had no effect on leaf mineral content (Mn, Fe, Cu, and Zn) or yield at two different sites in Brazil.[121] There was also no effect of absorption of exogenously applied Mn. Exogenous Mn application had no effect on yield of glyphosate-treated, GR soybeans, but it did enhance Mn and reduce Fe content in this study. No effects 0.86 kg ha^{-1} glyphosate sprayed once or twice on Mn content of both greenhouse- and field-grown GR soybean leaves (young and old) or seed (Figure 6).[80] There was no effect of glyphosate on yield in this study. The results of all of these studies indicate that glyphosate does not restrict the availability of micronutrients in glyphosate-treated, GR crops. Thus, the results of the three research groups that have reported glyphosate effects on mineral nutrition in GR crops are counter to those of nine other research groups.

Possible Interactions of Glyphosate with Fe Deficiency Chlorosis of GR Soybean. In many locations in IA, MN, ND, and some other U.S. states, soybeans may suffer iron-deficiency-chlorosis (IDC) when grown on wet calcareous

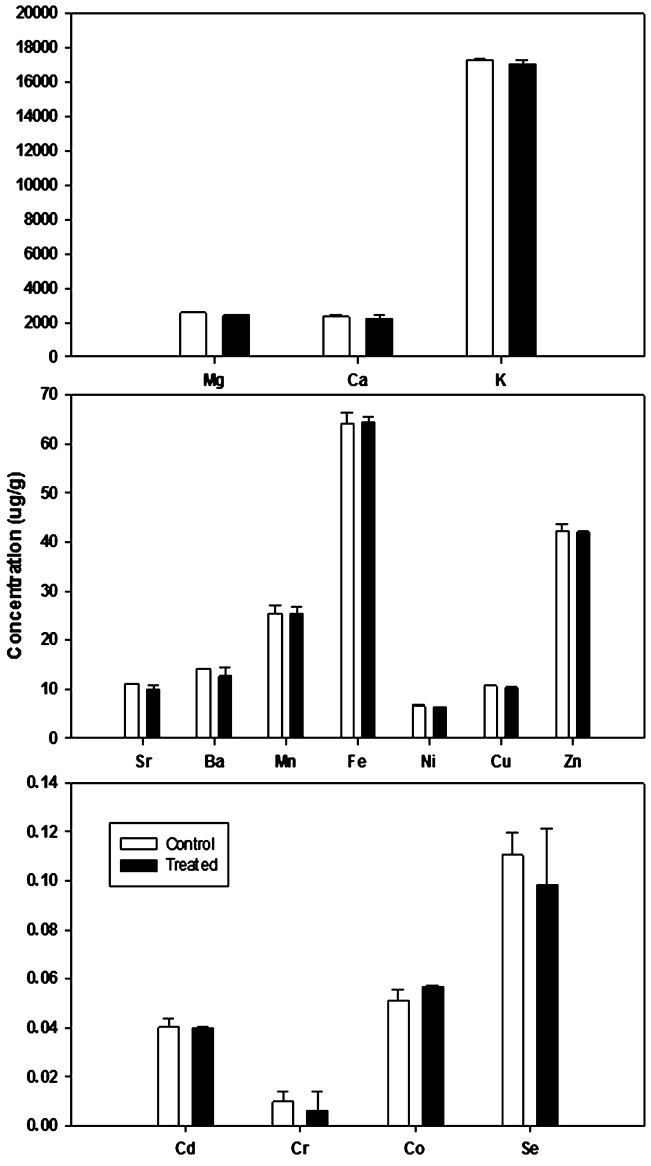

Figure 6. Effects of two, successive glyphosate treatments (0.86 kg ai h^{-1} at both 3 and 6 weeks after planting) on the metal content of mature seeds of field-grown GR soybean plants. Bars respresent 1 SE. There were no differences among any of the paired mean values at the 95% confidence level.[80].

soils.[122−124] Soybean cultivars vary widely in resistance to IDC, and many factors which influence soil moisture and bicarbonate levels interact with severity of IDC.[123,125] IDC can cause severe yield reduction on problem soils if cultivars are not highly resistant to IDC.[125−127] Because of the susceptibility of many soybean cultivars to IDC, growers with problem soils are advised to select chlorosis-resistant cultivars. Unfortunately, when GR soybeans were first developed, the cultivars which were initially transformed were not highly resistant to IDC, and many of the early high yielding GR soybean cultivars developed for normal soils were susceptible to IDC on wet calcareous soils. Soybean agronomists in states where IDC is prevalent now screen genotypes for resistance to IDC and report the results to growers so they can choose cultivars to match their soil IDC problems. Thus, GR soybean cultivars have been screened for susceptibility to IDC with and without glyphosate applications in many locations. Although this has not been reported in the literature, several scientists involved in soybean IDC screening confirm that based on their observations in chlorosis rating field plots, glyphosate causes no adverse interaction with iron deficiency in soybean (S.R. Cianzio, Iowa State University; J.H. Orf, University of Minnesota; T.C. Helms, North Dakota State University - Personal communications).

Possible Interaction of Glyphosate with Ni Phytoavailability. Although Duke et al.[80] found no effect of two applications of glyphosate on nickel content of leaves or seed (Figure 6) of GR soybean, another report notes that glyphosate use on GR-soybeans in a Brazilian study caused a significant reduction in plant N-fixation and a decline in leaf Ni.[14] Ni deficiency can reduce N-fixation. The foliar Ni levels, even in their controls, were far below normal soybean foliar Ni levels in other research. Ni is an essential element for all plants,[128] but in the U.S. Ni deficiency of significant consequence in the field has only been observed with some low Ni soils of the southeastern Coastal Plain where pecans suffered severe deficiency under some conditions of previous management which included raising soil pH which reduces Ni phytoavailability.[129] Legumes have a higher Ni requirement than nonlegumes because Ni is needed for biochemical processes in nodule bacteria, as well as for certain plant biochemistry. Unfortunately, Zobiole et al.[14] did not test application of foliar Ni fertilizer to confirm that the measured yield reduction actually resulted from Ni deficiency induced by glyphosate. Furthermore, the level of Ni in the Brazilian soil was not reported, so whether soil Ni deficiencies were involved cannot be determined. That glyphosate is directly toxic to some strains of *Bradyrhizobium japonicum* due the fact that their EPSPS is also sensitive to glyphosate is well-known (see section under Glyphosate Effects on Soil Microflora below), and this toxicity is not related to effects on Ni. Studies designed to address the interactions of glyphosate and Ni metabolism conducted on Ni-deficient soils with and without Ni supplementation would be useful in interpreting the results of Zobiole et al.[14]

Mineral Content in Compositional Equivalence Studies in GR Crops. There are numerous studies on the compositional (chemical and nutritional) equivalence of GR crops with GS crops, including mineral content, although the intent of these papers was to evaluate the effect of the transgene(s) on composition, rather than the effect of glyphosate treatment on GR crops. In most of the published studies no mention is made of whether glyphosate was used on the crop.[130−132] In other studies the glyphosate application to the GR crop is not completely described. For example, in Ridley et al.,[133] the timing of glyphosate applications in GR corn is given, but not the rates. In another study with GR corn[134] the only information provided for the glyphosate treatments was that they were made according to the label. A study with GR alfalfa states only that glyphosate was applied prior to each cutting.[135] More detailed information on the glyphosate applications is provided in a study with GR corn by Ridley et al.[136] For one set of trials, the GR corn received an application of 1.08 kg ha^{-1}, and in another ca. 0.85 kg ha^{-1} was used. The purpose of these studies was to provide data required by regulatory agencies to determine the effect of the transgenes on the composition of the harvested crop. No effects of the GR transgenes or glyphosate application on mineral content have been found in these field studies conducted under good laboratory practices that usually involved multiple years and locations. However, these studies lacked comparisons of

glyphosate-treated with untreated crops to allow evaluation of the glyphosate effect, independent of the genetic effect of the GR technology.

Summary of Glyphosate Interactions with Plant Mircronutrient Status. Clearly, glyphosate can have effects on mineral nutrition of GS plants through its herbicidal effects on plant roots and other parts of the plant. Published data on the effects of glyphosate on mineral nutrition of GR crops are contradictory. Three groups have claimed adverse effects on mineral nutrition in GR crops in peer-reviewed journals—the Zobiole et al. group,[12−15,17,19] Bellaloui et al.,[20] and Bott et al.[10] Others have made similar claims in nonpeer-reviewed venues.[111,112] The peer-reviewed results of nine laboratories[34,36,80,86,103,109,117−121] show no effect of glyphosate on mineral nutrition. These seemingly contradictory results could be entirely or in part due to differences in the soils, climatic conditions, and/or GR cultivars used. For example, one group of experiments is based almost entirely on studies with low pH soils using soybean varieties developed in Brazil and evaluated in greenhouse studies.[12−19] Rigorous field studies on different soil types (including those highly susceptible to inducing Mn or Fe deficiency in soybeans) are needed to resolve the issue of whether glyphosate might have adverse effects on mineral nutrition of GR crops. Considering the available data, growers are unlikely to need Mn fertilizers just because they use glyphosate on GR soybeans.[113]

GLYPHOSATE EFFECTS ON SOIL MICROFLORA

Soil microflora can influence the persistence of glyphosate and its metabolites in soil. Rhizosphere microflora can also influence uptake of soil minerals by crops. Evaluation of glyphosate effects on soil microorganisms requires knowledge of the direct effects of glyphosate and its metabolites on soil microorganisms as well as effects on microorganisms through processes mediated by plants on root symbionts and rhizosphere microorganisms. The determination of relevant environmental exposure concentrations needs to be compared to known response factors. Finally, short-term and long-term responses on processes and community structure need to be evaluated.

Glyphosate Toxicity to Microorganisms. As in plants, glyphosate blocks the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan in some bacteria and fungi through the inhibition of EPSPS, which also causes accumulation and excretion of shikimate-3-phosphate and hydroxybenzoic acids in sensitive microorganisms.[137,138] The sensitivity of bacterial EPSPS to glyphosate varies widely. Pollegioni et al.[42] divided microbial EPSPS into two groups: sensitive (Class I) and relatively insensitive (Class II). Class II includes *Agrobacterium* CP4 (the source of the GR-EPSPS transgene in most GR-cultivars) in which the resistance to glyphosate results from variations in the amino acid sequence of EPSPS. Concentrations required for 50% inhibition were 75 μM for *E. coli*, 174 μM for *Bacillus subtilis*, and 1100 μM for *Pseudomonas aeruginosa* EPSPS.[137] Moorman et al.[138,139] reported variation in susceptibility of strains of *Bradyrhizobium japonicum* to glyphosate: 1000 μM (169 mg L^{-1}) glyphosate produced 47% inhibition for strain 110, but only 12 and 19% inhibition for strains 123 and 138, respectively. Similarly, Hernandez et al.[140] reported *B. japonicum* strains ranging from sensitive to glyphosate (50% inhibition at 30 μM) to insensitive (50% inhibition at >1000 μM). The full range of resistance or sensitivity to glyphosate within the soil microbial community is not fully known. Addition of aromatic amino acids to bacterial cultures can partially or fully reverse the effects of glyphosate. Some fungi are also sensitive to glyphosate, with 50% inhibition of growth at concentrations of 5 to 50 mg/L (0.84−8.4 μM) in culture.[141]

Understanding the impact of glyphosate on soil microorganisms requires estimating concentrations to which the microorganisms are exposed. Multiple applications of glyphosate may occur in GR cropping systems. Glyphosate applied to foliage is rapidly translocated to roots and other metaboically active tissues.[22] Glyphosate is exuded from roots of treated plants into the rhizosphere,[142−147] but the resulting concentrations in the rhizosphere soil are difficult to document. Glyphosate applied to GS crops can be translocated to the roots and released initially in exudates and later from decaying tissues. As much as 15% of glyphosate applied to sensitive plants could be translocated to roots.[51,146] Similar patterns of translocation were seen in GR-corn roots.[148] Laitinen et al.[146] also showed movement of glyphosate from roots of treated plants to the soil, with the concentration of glyphosate reaching 0.07 mg kg^{-1} soil in the rhizosphere at four days after application.

Glyphosate may also alter the quantity and quality of root exudates. Kremer et al.[145] compared carbohydrate and amino acid exudation from roots of GR soybeans with or without glyphosate treatment in hydroponic culture. Amino acid exudation was increased by glyphosate, but carbohydrates (measured by an anthrone reaction) were not different. Glyphosate treatment of a GS soybean variety (Williams[82]) also resulted in increased carbohydrate exudation. The root exudation of shikimate-3-P and protochatecuic acid have not been examined, but exudation of these compounds might be expected from GS plants after glyphosate application, as glyphosate causes marked accumulation of these compounds in sensitive plants (e.g., Lydon and Duke[149]).

Effects on Soil Microbial Populations and Community Structure. The effects of glyphosate on microorganisms in soil have been extensively investigated using a variety of techniques. Two techniques that investigate the community level responses, microbial biomass and respiration, show either no effect or a temporary inhibition of respiration due to glyphosate applied at rates less than 50 mg kg^{-1}.[150−153] At glyphosate application rates above 50 and up to 1500 mg kg^{-1} soil, soil respiration was stimulated. The range of concentrations used in these studies resulted from different assumptions about the penetration of sprayed glyphosate into the soil (see Lancaster et al.[151]). The stimulatory effect of high glyphosate concentrations on soil respiration is partly attributed to microbial metabolism of glyphosate, but secondary effects due to N and P mineralization could also stimulate respiration. These concentrations of glyphosate seem sufficient to induce glyphosate toxicity; a hypothetical application of 50 mg kg^{-1} glyphosate soil at 25% gravimetric water content would result in a 1.18 mM aqueous concentration in a thin layer at the surface of the soil. However, rapid adsorption would reduce the concentration in the soil solution. A K_d of 50 would result in approximately 2% of the applied herbicide being present in the soil solution resulting in an aqueous concentration of approximately 24 μM glyphosate, which is sufficient to affect sensitive microbial species. Community level measures, such as respiration or total microbial biomass, are not sufficiently sensitive to detect changes in population or activity of small subpopulations.

Alternatively, glyphosate impacts on soil microorganisms can be assessed using measures of community structure and in long-term studies where cumulative impacts may be determined. Hart and Brookes[154] found no difference in microbial biomass, microbial respiration and N mineralization in soils after 19 years of annual glyphosate application compared to an untreated control soil. Busse et al.[155] compared Ponderosa pine (*Pinus ponderosa*) forest soils receiving glyphosate treatment for understory vegetation control to control treatments (understory cover at 25−100%). No glyphosate effects on soil respiration, N mineralization, or microbial biomass were found when these plots were evaluated after 9 to 13 years at each of the three sites. Powell et al.[156] compared a GR-soybean to a near isoline sensitive cultivar over four years in Ontario. Rates of soybean litter decomposition of the GR and conventional cultivars were nearly identical; however, glyphosate reduced litter decomposition on the soil surface, but not on buried litter. The ratios of fungal biomass to bacterial biomass in the litter were only occasionally different, with an increased ratio in the GR cultivars. Protists and nematode populations were not affected.

Effects on Rhizosphere Populations and Community Structure. The rhizosphere is comprised of the root surface and the immediate soil layer (2−5 mm) surrounding the root where microbial processes are driven by root exudation of simple and complex substrates, which include organic acids, flavonols, lignins, indole compounds, and amino acids.[157] The rhizosphere community includes root symbionts, pathogens, plant growth-promoting rhizobacteria, phosphate-solublizing bacteria, and microoganisms active in carbon and nitrogen cycling.[158] Significant amounts of carbon are exuded from growing roots, and rhizosphere populations may be exposed to glyphosate through leaching of glyphosate from the soil surface and root exudation of glyphosate.

Mijangos et al.[159] examined glyphosate effects on GS plants (triticale and peas) and their rhizosphere microbial communities. Ammonia concentrations increased in rhizosphere soil after glyphosate treatment compared to the control (no glyphosate, but clipped to remove above-ground biomass). Functional diversity of the rhizosphere microbial community was examined using a multiple substrate utilization test (Biolog Ecoplates) and genetic diversity by denaturing gradient gel electrophoresis of 16S-rDNA after PCR amplification. Community diversity and richness were reduced at the highest rate of glyphosate application in rhizospheres of killed GS pea and GS triticale, but not in soil from triticale grown alone. The magnitude of these differences was similar to the differences due to growing triticale alone or in combination with peas.

Several studies using different methods have examined the impact of glyphosate on the rhizosphere of GR crops. Glyphosate application to GR-soybean cultivars in the field in two growing seasons caused transient differences in dehydrogenase activity, β-glucosaminidase activity, β-glucosidase, and respiration.[16] These enzyme activities are broadly distributed in soil microorganisms, and the results suggest that broad spectrum toxicity did not result from glyphosate application. Subsequent studies reported increases in the ratio of Mn oxidizers/Mn reducers in response to glyphosate and decreases in IAA-producing rhizobacteria.[16] The magnitude of these responses increased as the glyphosate application rate increased up to a rate equivalent to 1.2 g ha^{-1}. Manganese oxidation ($Mn^{2+} + 1/2O_2 + H_2O \rightarrow MnO_2 + 2H^+$) reduces the solubility of manganese. The observation that glyphosate affects the ratio of Mn oxidizers/Mn reducers in the GR soybean rhizosphere led to suggestions that glyphosate reduced plant available Mn in soil and plant uptake of Mn.[69] However, the extent that this shift to a higher ratio of Mn oxidizers to Mn reducers has on the availability of Mn to plants was not determined. Manganese is most available in soil under reduced conditions and/or at low (<5.4) soil pH. A phylogenetically diverse group of both bacteria and fungi are capable of Mn oxidation,[160] but the cultural methods used to assess Mn oxidation or reduction potential[16] may not measure all the microorganisms capable of Mn transformation, or their *in situ* activity. Also, plant roots actively regulate their ability to obtain Mn from soils, upregulate Mn transporters, and secrete reducing materials which would release Mn from bound forms in the soil for plant uptake. Additional research is needed to investigate glyphosate-induced changes in Mn bioavailability in the rhizosphere.

Lupwayi et al.[161] reported reduced functional diversity (also using the multiple substrate utilization test) in response to two glyphosate applications to GR-canola. Hart et al.[162] found that rhizosphere populations of denitrifying bacteria and fungi were not affected by glyphosate application to GR-corn compared to GR-corn treated with conventional herbicides or a GS corn isoline treated with conventional herbicides.

Barriuso et al.[163] extracted bacterial DNA from GR corn rhizospheres after pre-emergence treatment with no herbicide, glyphosate, or GTZ (a mixture of the herbicides acetochlor and terbuthylazine). Pyrrosequencing of cloned 16S-rDNA showed that microbial community structure after glyphosate treatment more resembled the control (no herbicide) than the GTZ-treated community. Glyphosate reduced Actinobacteria relative to the untreated control and Proteobacteria were relatively unaffected. The GTZ treatment reduced microbial diversity relative to the glyphosate or no-herbicide treatments. In contrast, Lancaster et al.[52] showed a variable response of Actinobacteria populations to one or five applications of glyphosate to soil without a crop, while Proteobacteria were increased by glyphosate applications. The concentrations of microbial fatty acid methyl-esters (FAME) from gram-negative bacteria also increased, which is consistent with the increase in Proteobacteria populations.

Longer-term (3 year) studies identified three microbial groups dominating the GR corn rhizosphere in two fields in Spain: the Proteobacteria, Actinobacteria, and Acidobacteria.[164] Glyphosate was applied postemergence to GR-corn, and roots were sampled 7 days after glyphosate treatment and just prior to harvest. DNA extraction and sequencing provided a database that was screened for 16S-rDNA phylogenetic sequences. The abundance of these groups indicated little effect of glyphosate over three years (Figure 7). Analysis of the same data with a clustering procedure showed that the rhizosphere community was most affected by year and field and least affected by time of sampling and herbicide. Acidobacteria increased over time in both fields (Figure 7), while Actinobacteria tended to decrease.

Lane et al.[165] also used FAME biomarkers to examine the effects of two postemergent glyphosate applications to GR-soybeans grown in soil with and without a history of previous glyphosate use. At 7 days after application, total FAME (an indicator of microbial biomass) was reduced in both soils. Nonmetric multidimensional scaling of the FAME data showed a significant effect of the soil (history vs no-history) on community structure, but no effect of application or sampling times on community structure. The ratio of fungal to bacterial biomass was also unaffected. The decrease in microbial biomass

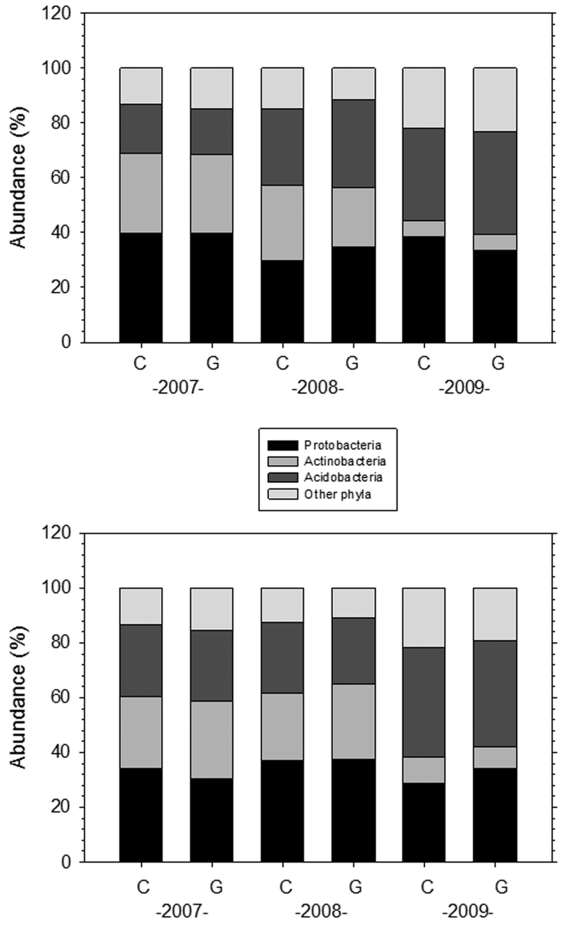

Figure 7. Eubacterial phyla (16S-rDNA sequence abundance) recovered from GR-corn rhizosphere treated with glyphosate (G) or without glyphosate (C) in two fields (upper and lower panels). Sampling was 7 days after glyphosate application. Drawn from data from Barriuso et al.[164]

at 7 days after application does not support the conjecture that glyphosate treatment increases root exudation. Weaver et al.[54] also used FAME analysis to compare rhizosphere and bulk soil community structures after glyphosate application to GR-soybean in the field. After the second in-season glyphosate application, the community structure of the bulk soil differed from that of the rhizosphere, but two previous applications of glyphosate had no effect on FAME. The same study included two fungal FAME biomarkers (16:1 ω5c and 18:2 ω6c), and these were not affected by the glyphosate treatments. The 16:1 ω5c (hexadecenoic acid) content is a biomarker for arbuscular mycorrhizal fungi,[166] while 18:2 ω6c is a more broad fungal marker, including *Rhizoctonia solani* and *Fusarium oxysporum*.[167]

Nodulation and N-fixation. Zablotowicz and Reddy[168] summarized the effects of glyphosate on soybean nodulation and N fixation. GR soybeans treated with glyphosate had reduced nodulation, as well as delayed N fixation, plant biomass accumulation, and N fixation, but the severity of these effects was dependent upon several factors. These included when glyphosate was applied to the soybean, the number of glyphosate applications, the glyphosate formulation, and the GR-soybean cultivar. Powell et al.[169] compared nodule number and mass in six GR and three near isoline GS soybean cultivars in the absence of glyphosate. Significant differences in nodulation were found among the cultivars, but these were not related to glyphosate resistance. Concentrations of glyphosate in nodules and roots of soybeans were low (<200 ng g^{-1} nodule tissue), although shikimate and hydroxybenzoic acids were present in three-to 4-fold greater concentrations, indicating inhibition of *B. japonicum* EPSPS.[168] Among strains of *B. japonicum*, glyphosate tolerance in culture was correlated with N fixation in excised nodules (acetylene reduction assays).

Multiple field studies show no effect of glyphosate on GR-soybean yield.[170] Using differences in natural abundance of ^{15}N, Bohm et al.[171] estimated the percentage of soybean N derived from fixation to be 80% for GR soybean without glyphosate, 57% for the same cultivar with one glyphosate application, and 66% after two applications. Yield was not affected, and Bohm et al.[171] suggested that the glyphosate-treated soybeans obtained more reduced N from the soil. *B. japonicum* growth in culture is reduced by glyphosate, but *Rhizobium* spp. degrade glyphosate when glyphosate toxicity is alleviated with aromatic amino acids.[44] These effects on nodulation and N fixation may be due in part to the inhibitory effects of glyphosate on *B. japonicum*, but may also be related to GR cultivar responses to glyphosate. Additional evidence of cultivar variability was found in a field study using 20 GR soybean cultivars with and without glyphosate applied at four combinations of rates and timings.[172] Of the 20 cultivars, 9 showed no difference in nodule biomass compared to the unsprayed treatment. Nodulation was reduced by as much as 61% for one cultivar. One GR cultivar, BRS 244 RR, which had no glyphosate effect on nodule biomass in this study, was reported to have reduced nodulation after glyphosate application in a subsequent study.[17] The survival of *B. japonicum* in soil without plants was not affected by concentrations equivalent to 1X or 10X field application rates of glyphosate.[139] Selection or construction of GR *B. japonicum* would be an effective strategy for alleviating negative effects on nodulation and N fixation.

The glyphosate metabolite AMPA can temporarily reduce chlorophyll content (causing yellowing or chlorosis) and photosynthesis in GR soybeans, particularly after foliar applications of AMPA at 1.0 kg ha^{-1} or high rates of glyphosate.[104,108] This rate was chosen to represent the complete metabolism of a glyphosate application to AMPA. This rate of AMPA did not affect nodulation or nitrogenase activity, suggesting that *B. japonicum* is less sensitive to AMPA than soybeans. The responses of GS cultivars were similar to the GR cultivars, which are explained by the fact that AMPA does not affect EPSPS. The mechanism of action of AMPA is unknown.

Arbuscular Mycorrhizae. Arbuscular mycorrhizal fungi are obligate symbionts that transfer mineral nutrients to their plant hosts.[173,174] Savin et al.[175] evaluated glyphosate effects on arbuscular mycorrhizal fungi (AMF) colonization of GR cultivars of cotton, corn and soybean grown in soil under greenhouse conditions. AMF colonization of roots was not affected by glyphosate, and neither were acid nor alkaline phosphatase soil enzyme activities. Similar results were obtained by Knox et al.[176] Other research has shown that in the tripartite symbiosis of mycorrhiza, rhizobium, and soybean, no adverse effects of glyphosate use on GR cultivars was observed.[169] These studies indicate that effects of glyphosate on plant mineral nutrition through effects on AMF are unlikely.

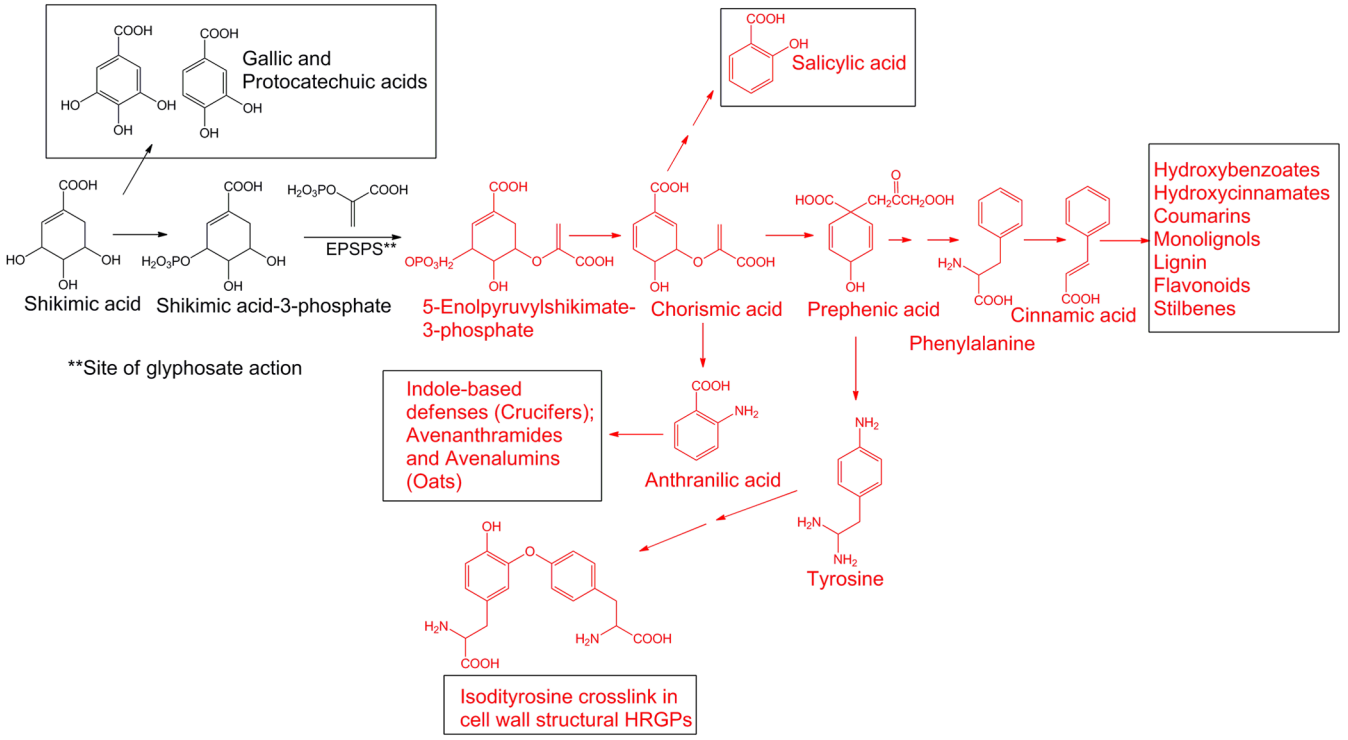

Figure 8. Possible effects of glyphosate treatment of glyphosate-senstive plants on shikimic acid pathway metabolites considered to be important in defense. Products of the shikimic acid pathway involved in plant defenses are outlined by boxes. Metabolites and metabolic groups in red have been demonstrated or are hypothesized to be reduced in GS plants after glyphosate treatment. Of these, only isoflavonoid phytoalexins from bean[189] and soybean[190] and lignin deposition[191] in bean have been examined for effects of glyphosate (and only in GS plants). Those in black are expected to increase after glyphosate treatment. Protocatechuic acid has been demonstrated to increase in GS plant tissue after glyphosate treatment.[149] **EPSPS: 5-enolpyruvylshikimic acid-3-phosphate synthase, the site of glyphosate action.

EFFECTS OF GLYPHOSATE ON PLANT DISEASE IN GLYPHOSATE-RESISTANT CROPS

Plants use a variety of preformed and postinfection-induced defenses to resist pathogens. These include phenolic compounds which are considered to be major components of defense across the plant kingdom.[177−179] Phenolic compounds may act in defense as preformed antibiotics, pathogen-induced phytoalexins, or as structural barriers in the form of lignin. Thus, it is not surprising that any alteration in phenolic metabolism may have an impact on the expression of disease. For example, treatment with inhibitors of phenylalanine ammonia lyase have been shown to enhance disease susceptibility (e.g., Holliday and Keen[180]), while treatment with compounds such as microbial elicitors[181] or even herbicides such as the protoporphyrinogen oxidase (PPO) inhibitor lactofen can stimulate accumulation of phenolic compounds and enhance disease resistance.[182]

Because glyphosate inhibits EPSPS, a key enzyme in the shikimic acid pathway, it also inhibits the biosynthesis of phenyalanine-derived phenolic compounds and should also result in lack of synthesis of salicylic acid from isochorismate.[183] Hence, this herbicide may enhance susceptibility to diseases in plants that are susceptible to glyphosate. In addition to phenolic compounds, glyphosate should also prevent synthesis of anthranilic acid, an intermediate needed for the synthesis of the indole-based glucosinolates and phytoalexins in crucifers[184,185] and the avenalumin phytoanticipins[186] and avenanthramide phytoalexins[187] in oats. Finally, many plants respond to infection by strengthening their cell walls with hydroxyproline-rich glycoproteins.[188] Because these proteins contain a significant amount of tyrosine, and formation of isodityrosine cross-links is important in cell wall reinforcement, it would seem likely that glyphosate may also impact this aspect of plant defense. However, the effect of glyphosate on these non-phenylpropanoid based defenses has not been reported. A summary of possible effects of glyphosate on plant defenses derived from the shikimic acid pathway in shown in Figure 8. Based on both known and potential effects of glyphosate on disease defense compounds, it is not surprising to find reports in the published literature that show the disease-enhancing effects of glyphosate on GS plants (e.g., reviewed in Johal and Huber[69] and Duke et al.[192]).

Fungal and Oomycete Diseases. *Research with GS Plants and Fungal Diseases.* The effect of glyphosate on disease resistance of GS plants was initially reported by Keen and co-workers[190] in 1982. They reported that treatment of GS soybean hypocotyls with glyphosate decreased the resistance to *Phytophthora megsaperma* and reduced the accumulation of the isoflavonoid phytoalexin glyceollin. In a subsequent study, Ward [193] confirmed the results of Keen et al.[190] and also showed that glyphosate also reduced the efficacy of metalaxyl, a fungicide specific for oomycetes.

The resistance breaking effect of glyphosate has also been tested in two GS bean pathosystems. Pretreatment of bean hypocotyls with glyphosate resulted in only a subset of the plants becoming more susceptible to infection with an incompatible (avirulent) race of *Colletotrichum lindemuthianum*.[189] The increase in susceptibility was associated with a decrease in phytoalexin production, but it is important to note that the response was not uniform. A much different result was

found in the bean−*Pythium* interaction.[194] In this case, treatment with glyphosate greatly increased susceptibility of roots to *Pythium*. This change in host reaction was associated with reduced accumulation of phenolic phytoalexins and deposition of lignin.[191]

Although treatment of GS plants with glyphosate can result in increased susceptibility to pathogens, it is important to know if GR crops can be predisposed to susceptibility by treatment with glyphosate. Biochemically, it would seem unlikely that GR plants would become more susceptible after glyphosate treatment, but is that the case? Johal and Huber[69] and Kremer and Means[195] reviewed glyphosate effects on GS and GR cultivars and suggested that fungal root diseases were increased by the adoption of GR-cultivars and the increased use of glyphosate.

GR Soybean and Sclerotinia Stem Rot (White Mold). Several studies have addressed whether or not GR plants are more susceptible to disease, with much of the work focusing on GR soybeans. Lee et al.[196] tested two near-isogenic lines of soybean GL2415 (GS) and GL2600RR (GR) for susceptibility to *Sclerotinia sclerotiorum*, the cause of the Sclerotinia stem rot or white mold disease. Using a detached leaf assay, there was no significant difference in lesion development in nontreated GL2415 as compared to GL2600RR. The formulation blank for the glyphosate product used in the work also had no effect on disease. Most important was the observation that treatment of GL2600RR with three different rates of glyphosate did not increase the severity of disease in that line as compared to the untreated controls for both GL2600RR and the GS line GL2415. Thus, the conclusions for this work were that the GR gene had no impact on disease and treatment of the GR plants with glyphosate did not enhance susceptibility. Nelson et al.[197] provided further evidence that the GR trait did not impact host reaction to *S. sclerotiorum* in field studies. Comparing four lines of soybean that were near-isogenic for the GR trait, they found that, with one exception, the glyphosate resistance trait had no effect on disease reaction. Glyphosate treatment of two GR lines increased disease as compared to the untreated, while the opposite effect was observed with two others. However, the two GR lines that showed increased disease after glyphosate treatment and their near-isogenic lines were significantly more susceptible to *Sclerotinia* as compared to the two other lines in which glyphosate had no effect on disease. Interestingly, treatment of the two most white mold susceptible GR lines with another soybean herbicide (thifensulfuron) resulted in enhanced disease development comparable to the plants treated with glyphosate. Lactofen, a PPO inhibitor known to induce resistance to *S. sclerotiorum*,[182] was able to reduce disease severity in all lines regardless of the presence or absence of the GR gene. Perhaps most significant was the observation that glyphosate treatment of GR lines had no effect on yield regardless of the amount of disease.

Lee et al.[198] also addressed the issue of cultivar differences in Sclerotinia stem rot susceptibility by examining management options. This work further illustrated that issues related to greater susceptibility of GR soybeans was not related to glyphosate resistance trait, but rather to the susceptibility of the cultivars that were used for transformation.

A lack of impact on defense responses in GR soybean was supported by analysis of glyceollin accumulation in resistant as compared to susceptible plants.[199] Using silver nitrate as an elicitor, glyphosate treatment did not reduce the accumulation of glyceollin.

GR Soybean, Fusarium, and Sudden Death Syndrome. Glyphosate application to some GR-soybean cultivars increases *Fusarium* spp. infection of roots in greenhouse experiments[16,195] and under field conditions.[195] For example, *Fusarium* spp. colonization increased from 20 to 30 infections per 100 cm of untreated soybean root to as little as 30 infections or as much as 120 infections per 100 cm root, depending upon glyphosate dose and soybean growth stage and cultivar.[16] In the same studies, decreases in populations of *Pseudomonas* spp. and indole acetic acid (IAA)-producing bacteria, as well as a reduction in the ratio of Mn-reducing to Mn oxidizing microorganisms were observed. Fluorescent *Pseudomonas* populations in the GR-rhizosphere were decreased by glyphosate application and negatively correlated with *Fusarium* root colonization.[195] These *Fusarium* infections of soybeans roots developed from soil-borne inoculum, and the species distribution of *Fusarium* was not determined.

The mechanisms of these glyphosate-mediated increases in *Fusarium* root infection in GR soybeans are not established. Results of studies on translocation of ^{14}C-glyphosate from treated leaves into roots and rhizospheres indicate that beneficial microorganism-inhibiting glyphosate concentrations could occur. Kremer et al.[145] showed that root exudates in general increased from glyphosate-treated plants grown in soil-free conditions. However, these studies did not establish that these root exudates specifically stimulated the growth of *Fusarium* spp. Still, glyphosate-mediated changes in quantity and quality of root exudates into the rhizosphere has not been sufficiently evaluated as an influence on plant disease.

The effects of glyphosate and glyphosate resistance in relation to sudden death syndrome (SDS), in soybean caused by *Fusarium virguliforme* (formerly *Fusarium solani* f. sp. *glycines*) has also been examined. Sanogo et al.[200] reported on the effects of glyphosate and two other soybean herbicides (lactofen and imazethapyr) on SDS response in two GR soybean lines (Pioneer 9344 and Asgrow 3701) and one GS line (BSR101). Two of the lines, Pioneer 9344 and BSR 101, are susceptible to SDS while the other line was noted by the authors as having "above average tolerance". In growth chamber tests, the foliar symptom severity of glyphosate-treated plants was no different than the untreated control or plants treated with imazethapyr. Lactofen treatment decreased SDS severity. In a greenhouse test, the severity of foliar and root symptoms of SDS was increased by both glyphosate and imazethapyr (with the exception of foliar severity in BSR 101 in which glyphosate treatment resulted in no difference from the control). These results suggested that the glyphosate resistance trait did not impact SDS response and that all three lines reacted to infection by *Fusarium* in a similar manner after herbicide treatments.

Sanogo et al.[201] later reported on the field reaction of the same three soybean lines to *F. virguliforme* and treatment with the same three herbicides plus acifluorfen. They examined both foliar symptoms and frequency of *Fusarium* isolation from roots, and found no significant cultivar-herbicide interaction. There was also a lower amount of disease in the more resistant line regardless of herbicide type as compared to the two susceptible lines, and treatment with glyphosate, acifluorfen and imazethapyr all increased disease severity in susceptible lines as compared to controls. Lactofen, in general, had no effect on disease severity compared to the controls. The authors concluded that there was no change in host resistance to SDS as a result of glyphosate treatment.

The effects of glyphosate treatments on SDS were examined in ten GR soybean lines from a variety of maturity groups.[202] In work similar that of Sanogo et al.,[200,201] Njiti et al.[202] reported no effect of glyphosate treatment on yield, foliar symptoms, or root infection. The overall conclusion from this study is that the host genotype, and not glyphosate resistance or treatment with glyphosate, was the most important factor in determining the reaction of a cultivar to SDS.

Lévesque et al.[203] found that glyphosate sprayed on mixed populations of weed species caused increased *Fusarium* spp. infection in some weed species, but not in others. The number of colony-forming units of *Fusarium* spp. per gram of dried soil increased after application of glyphosate, but GS crops (corn, pea, cucumber, and bean) subsequently grown on in the field were not affected. Powell and Swanton[204] concluded that there was insufficient evidence to prove a link between glyphosate and plant diseases associated with *Fusarium* spp.

GR Soybean and Rhizoctonia solani. Harikrishnan and Yang et al.[205] examined the effect of glyphosate and other herbicides on reaction of BSR 101 (GS) and Pioneer 93B01 (GR) to *Rhizoctonia solani*. In greenhouse studies, the severity of *Rhizoctonia* infection in autoclaved soil was not increased by glyphosate in Pioneer 93B01 compared to the inoculated control. In fact, statistically similar levels of severity were also observed after imazethapyr treatment. In nonautoclaved soil, glyphosate actually reduced the severity of *Rhizoctonia* as compared to plants that were inoculated but not treated with a herbicide. In two years of field trials, glyphosate treatment of Pioneer 9344 resulted in no difference in response to *Rhizoctonia* infection based on shoot dry weight, *Rhizoctonia* severity and plant stand.

Effect of GR Soybean in Rotation with Cereals. GR soybeans are a rotation crop with cereals, and recent reports have suggested that glyphosate treatment of soybeans increases the occurrence of Fusarium head blight of wheat and barley.[206,207] Because of these observations, Bérubé et al.[208] tested the effects of tillage and glyphosate treatment of GR soybean on Fusarium head blight development in a subsequent planting of wheat and barley. There was no measurable effect of treating GR soybeans with glyphosate on development of head blight and accumulation of mycotoxins in barley or wheat.

Other GR Crops. In sugar beet, GR varieties were tested for the effects of glyphosate treatment on expression of disease caused by *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *betae*.[209] Inoculation with *R. solani* isolate R-1411 (AG-4) resulted in comparable amounts of disease in both B4RR and H16 whether or not they were treated with glyphosate or a surfactant. However, inoculation with *R. solani* R-9 (AG-2-2) revealed that glyphosate treatment resulted in increased disease in B4RR as compared to H16. This suggests that glyphosate did have a negative effect on resistance in B4RR. Inoculation with *F. oxysporum* isolate Fob13 resulted in increased disease in glyphosate-treated B4RR and H16 as compared to nontreated controls. There was no effect of glyphosate treatment on infection of the two sugar beet lines by *F. oxysporum* isolate F19. In a two year field study with GR sugar beet, Barnett et al.[210] reported that glyphosate had no effect on expression of Rhizoctonia crown and root rot in four GR lines (Hilleshög 9027RR, Hilleshög 9029RR, Hilleshög 9028RR and Crystal). They also reported that glyphosate treatments did not impact efficacy of the fungicide azoxystrobin. Using field and greenhouse evaluations, a follow-up study by Barnett et al.[211] confirmed that glyphosate treatment of GR lines had no effect on reaction to Rhizoctonia. Their recommendation to growers was to use GR sugar beet varieties with the greatest amount of *Rhizoctonia* resistance.

Two wheat lines that were near-isogenic for glyphosate resistance were tested for the effect of glyphosate on disease caused by *Rhizoctonia oryzae*, *R. solani*, *Pythium ultimum* and *Gaeumannomyces graminis* var. *tritici*.[212] The GR lines were not more susceptible to any of these pathogens than the lines from which they were derived. Furthermore, glyphosate application to the GR lines did not increase disease severity. However, this study reported that volunteer GS wheat, if killed by a foliar treatment with glyphosate resulted in increased infection by *R. solani* and *G. graminis* var. *tritici,* possibly as a result of increased amounts of pathogen inoculum produced in the crop residue.

The reaction of GR cotton seedlings to *Rhizoctonia solani* after treatment with several pre- emergent herbicides and glyphosate as a foliar treatment was tested in field and greenhouse experiments.[213] Glyphosate applied at the cotyledon or four leaf stage of GR cotton reduced Rhizoctonia infection of hypocotyls in the field. In greenhouse studies, several pre-emergent herbicides predisposed cotton seedlings to greater hypocotyl infection by *R. solani*, but subsequent application of glyphosate did not increase severity of the disease. Baird et al.[214] found that four varieties of GR cotton (PM 1220, DPL 5690, DPL 5415, and DPL 50) had similar seedling stand count, height, and dry weight when compared to GS varieties from the same lineage group, regardless of glyphosate application. When differences did occur, no consistent trends could be determined within the lineage groups tested.

Glyphosate as a Plant Protectant. Glyphosate was shown to have both preventive and curative activities against both stripe rust (*Puccinia striiformis* f. sp. *tritici*)[215,216] and leaf rust (*Puccinia triticina*) on GR wheat.[215,217] In these cases, it appears that glyphosate is acting directly as a fungicide. Some efficacy against *Phakopsora pachyrhizi*, the cause of Asian soybean rust, was reported in both greenhouse[215] and in the field on GR soybeans.[216] Tuffi Santos at al.[218] showed that glyphosate reduced the severity of rust caused by *Puccinia pdisii* on *Eucalyptus grandis*. They found that there was a systemic effect of glyphosate on rust development as illustrated by reduced urediniospore germination and appressorium formation on tissues that were not directly treated with the herbicide.

Similar to soybean, glyphosate has recently been reported to protect GR alfalfa against the rust *Uromyces striatus* when applied prior to or up to 10 days after inoculation.[219] In this study, glyphosate was found to provide some protection against *Colletotrichum trifolii* and *Phoma medicaginis*. These latter two results are interesting as these pathogens, unlike biotrophic rusts, are hemibiotrophic and necrotophic in their attack of their hosts.

Bacterial Diseases. *GS Soybean and Bacterial Blight.* Holliday and Keen[180] examined the effect of glyphosate on the response of GS soybean leaves to the bacterial pathogen *Pseudomonas syringae* pv *glycinea*. In this case, the effect of glyphosate on resistance was less conclusive. Although glyphosate treatment significantly decreased glyceollin accumulation, it had no effect on the expression of the hypersensitive response. Glyphosate treatment also resulted in only a relatively small increase in bacterial growth in the treated plants. This suggests that in GS plants resistance to bacterial blight is not greatly reduced after treatment with glyphosate.

GR Soybean and Bacterial Pustule. Several hundred GR soybean lines were screened for resistance to bacterial pustule, caused by *Xanthomonas axonopodis* pv *glycines*.[220] The authors report that resistance to the disease occurs in GR soybeans, but that not all genotypes were resistant. Although they did not test the effect of glyphosate on the host response to Xanthomonas, the authors did recommend that growers assess the risk for this disease and plant resistant cultivars when the disease is likely to occur.

GR Corn and Goss's Wilt. Goss's wilt and leaf blight of corn, caused by the gram positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis*, has increased over the last 5 years in corn-producing states,[221−225] as has increased planting of GR corn (Figure 1). There are some logical explanations for the recent increase in the occurrence of Goss's wilt and leaf blight in corn growing areas that do not implicate the use of GR corn or glyphosate. These include continuous corn production over many years with minimal tillage. Both of these practices will allow the buildup of pathogen inoculum over time, and reduced tillage practices allow the pathogen to survive. Reduced tillage practices that reduce residue decomposition will also increase pathogen inoculum, although one of the perceived benefits of GR crops has been the ability to manage weeds in reduced tillage.[226,227] Another factor that may contribute to increased disease development are reduced efforts to select for resistant hybrids and/or failure to promote resistant hybrids by seed companies. Finally, weather events (such as early season hail damage) will promote infection in even the most resistant hybrids.[221,228] Changes in *C. michiganensis* subsp. *nebraskensis* genotypes might also be a factor, but further work is needed to determine if this has occurred.[222] There was no mention in any of the recently published reports that the GR trait or glyphosate application is a contributing factor to the increase in Goss's wilt. The report by Ruhl et al.[225] noted that the first Indiana finds were on both field corn and popcorn. Since popcorn is not GR, this would further implicate other factors in the recent outbreaks of the disease. Considering that most corn produced in the US is now GR[229] (Figure 1), it is likely that inoculum buildup and use of GR corn that is not resistant to Goss's wilt are the reasons for increases in this disease. In addition, there are no reports in the published literature that suggest that glyphosate resistance or treatment of GR varieties with the herbicide will increase the risk of other diseases in this crop.

Soybean Cyst Nematode. Yang et al.[230] examined the effect of glyphosate on soybean cyst nematode (SCN, *Heterodora glycines*) infection of the GR and SCN-resistant variety Countrymark 316. Greenhouse tests demonstrated no effect of glyphosate on SCN development on this genotype as compared to untreated controls. Noel and Wax[231] compared the reactions of GR soybean lines DR 320 (SCN susceptible) and DSR 327 (SCR resistant) to glyphosate treatment and inoculation with *H. glycines*. They reported that glyphosate did result in increased numbers of the nematode on the susceptible line, but not the resistant line. Even with the increase in nematode populations, there was no impact on yield. This study, like those with other soybean diseases, suggests that genotypic resistance or susceptibility, rather than glyphosate resistance, is the most important factor related to disease severity.

Summary of Glyphosate and Disease Resistance. Although it is clear that glyphosate does increase severity of disease on GS plants, the published evidence for its effects on GR plants presents a different story. Overall, it appears that in GR crops the baseline disease resistance or susceptibility of the host plant, not the presence of the glyphosate resistance gene or treatment with glyphosate, is the major contributor to susceptibility.

YIELDS OF GLYPHOSATE-RESISTANT CROPS

In the U.S., GR soybeans, cotton, and corn were introduced in 1996, 1997, and 1998, respectively. Adoption of the crops has been rapid and overwhelming, with more than 90% of soybeans, ca. 80% of cotton, and about 70% of corn currently grown being GR (Figure 1). After the introduction of GR sugar beets in 2008, the adoption rate was essentially 100% in 2009.

Thus, one might expect that if there were any significant mineral nutrition and/or disease problems with these crops, the problems would be manifested in yield reductions and farmer dissatisfaction. Yield data from the years before introduction of GR crops, continuing to the present show that the same yield trends before introduction continued after introduction (e.g., Figure 9). While there could be isolated pockets of adverse effects of glyphosate on GR crops that would be masked by their general success, such cases have not been conclusively documented. There were initial concerns with transgenic crops in general that there would be "yield drags" due to factors not associated with disease or mineral nutrition, but to suboptimal cultivars and potential pleiotrophic effects of the transgenes.[232] These problems have not materialized. To summarize, yield data for crops that are now predominantly GR cultivars do not support the view that there are significant mineral nutrition or disease problems with GR crops.

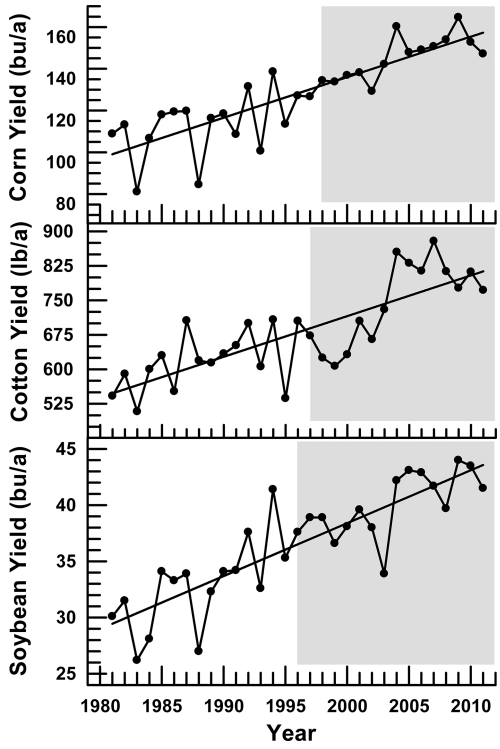

Figure 9. U.S. yields of the three crops over the past 30 years that are now grown mostly as GR cultivars. The shaded area represent the years since the introduction of each GR crop. Data are from the USDA, National Agricultural Statistics Service Data and Statistics Web site: http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/ (accessed September 12, 2012). GR crop adoption rates can be seen in Figure 1.

Scientific accounts about increased plant disease and mineral nutrition problems in GR crops are based on publications from a limited number of researchers. In the context of the entire body of relevant science, the significance of these reports is questionable. Still, considering the enormous importance of and reliance on GR crops and glyphosate, there has been a paucity of publically funded research into potential problems with this weed management technology. Farmers have generally embraced this technology, so that there has been no widespread call for studies of potential problems with GR crops other than those associated with GR weeds, a growing problem that is well documented. Furthermore, publication of negative (no effect) results is generally unattractive to journals, and, therefore, to scientists whose success depends on publications. So, the "no effect" papers that have been published may not represent all such data that have been generated. Reports of significant adverse effects of glyphosate on mineral nutrition and diseases of GR crops are perplexing in light of the considerable body of literature and yield data that contradict such claims. Nevertheless, there might be effects of glyphosate in GR crops on mineral nutrition and/or disease under particular but uncommon conditions (e.g., specific soil, environmental conditions, particular GR crop cultivars, and/or glyphosate formulations).

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Notes

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